


Formation of chondrospheres using MicroTissues 3D Petri dishes

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Magnetic levitational bioassembly of 3D tissue construct in space

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Detailed protocol

The tissue spheroids were formed using MicroTissues 3D Petri dish micro-molds (Sigma Aldrich, cat.# Z764019-6EA, 81 circular wells 800µm x 800µm) according to manufacturing protocol.

Prepare 2% agarose solution in PBS using microwave oven;
Allow molten agarose to cool to about 60-70°C;
Pipette 450 µl of molten agarose into micro-molds for casting MicroTissues 3D Petri dishes;
After the agarose had gelled (~ 4 min), carefully flex the micro-molds to remove MicroTissues 3D Petri dishes;
Transfer MicroTissues 3D Petri dishes to a 12-well culture plate;
To equilibrate MicroTissues 3D Petri dishes, add cell culture medium (2.5 mL/well);
Incubate for 20 minutes or longer;
Trypsinize cells, count and prepare cell suspension with concentration of 3.4×10^6 per milliliter;
Remove culture medium surrounding outside of MicroTissues 3D Petri dishes;
Seed 190 µl of cell suspensions into MicroTissues 3D Petri dishes;
Incubate for 40 minutes or longer;
Add an additional culture medium into each well of 12-well plate to the outside of MicroTissues 3D Petri dishes;
Incubate the 12-well plate containing MicroTissues 3D Petri dishes at 37°C in a humidified atmosphere with 5% CO₂ for 2 days;
To harvest the spheroids from the micro-wells carefully squirt culture medium into the MicroTissues 3D Petri dishes using a micro-pipette;
Draw the spheroids up into the micro-pipette.

Reagents and Materials:

MicroTissues 3D Petri dish micro-molds (Sigma Aldrich, cat.# Z764019-6EA, 81 circular wells 800µm x 800µm);
Agarose (Helicon, cat.# Am-0710-0.1);
Dulbecco's modified Eagle's medium, DMEM (Gibco, cat.# 12491-015);
Fetal bovine serum, FBS (Gibco, cat.# 16000-044);
Dulbecco's phosphate buffered saline, PBS (Gibco, cat.# 18912-014);
Antibiotic-antimycotic (Gibco, cat.# 15240-062);
Trypsin/EDTA (Gibco, cat.# 25200-114);
L-glutamine (Paneco, cat.# F032);
12-well cell culture plates (Corning, cat.# 3512).

How to cite: (Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Koudan, E. , Parfenov, V. , Demirci, U. and Mironov, V. (2021). Formation of chondrospheres using MicroTissues 3D Petri dishes. Bio-protocol Preprint. bio-protocol.org/1416.
2. Parfenov, V. A., Khesuani, Y. D., Petrov, S. V., Karalkin, P. A., Koudan, E. V., Nezhurina, E. K., Pereira, F. D., Krokhamal, A. A., Gryadunova, A. A., Bulanova, E. A., Vakhruhev, I. V., Babichenko, I. I., Kasyanov, V., Petrov, O. F., Vasiliev, M. M., Brakke, K., Belousov, S. I., Grigoriev, T. E., Osidak, E. O., Rossiyskaya, E. I., Buravkova, L. B., Kononenko, O. D., Demirci, U. and Mironov, V. A. (2020). Magnetic levitational bioassembly of 3D tissue construct in space . Science Advances 6(29). DOI: [10.1126/sciadv.aba4174](https://doi.org/10.1126/sciadv.aba4174)

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